Wakefulness: **An eye-opening perspective on orexin neurons**

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Orexin-containing neurons regulate wakefulness, and loss of orexin produces narcolepsy. Recent studies of mice lacking orexin neurons have shown that these cells also play essential roles in the control of feeding and energy balance.

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Predation, starvation and hypothermia are the leading causes of death for mice living in the wild. With little body fat, mice and many other animals often need to spend much of their active period watching for predators, searching for food and staying warm. This requires the coordination of systems that regulate feeding, body temperature and autonomic functions with those that govern the timing of alertness. Until recently, little was known about the control of these functions, but recent observations on the role of hypothalamic neurons containing orexins have demonstrated how one type of neuron may influence many physiologic functions.

Orexin-A and orexin–B — also known as hypocretin-1 and hypocretin-2 — are small neuropeptides produced by neurons in the lateral hypothalamus [1,2]. Orexin-containing neurons heavily innervate brain regions involved in the control of wakefulness, appetite, thermoregulation and autonomic control [3,4], and orexins have excitatory effects on post-synaptic neurons [2,5] (Figure 1). Orexin neurons are predominantly active during wakefulness [6], and injection of orexin-A into the lateral ventricles increases wakefulness, food intake, body temperature and sympathetic activity [1,5,7,8].

Although the orexins mediate many functions, narcolepsy is the most striking abnormality in animals with abnormal orexin signaling. Orexin knockout mice and dogs with orexin receptor mutations have many signs of narcolepsy and have provided key insights into the neurobiology of narcolepsy in humans [4,9]. People with narcolepsy have chronic, sometimes severe daytime sleepiness that typically begins in young adulthood and is often accompanied by episodic intrusions of rapid eye movement (REM) sleep-like phenomena into wakefulness. Vivid dreams and muscular paralysis occur during REM sleep, and people with narcolepsy may have dream-like hallucinations or sudden episodes of muscular paralysis or weakness known as cataplexy.

Orexin signaling is probably abnormal in people with narcolepsy because they have a marked reduction in the number of hypothalamic orexin neurons [10,11], and the concentration of orexin in their cerebrospinal fluid is usually very low [12]. Unlike the mouse and dog models, human narcoleptics rarely have mutations in the genes coding for orexin or its receptors [10]. Most likely an inflammatory, possibly autoimmune, process kills the orexin neurons in young adulthood. Whatever the cause, the loss of orexin from the brains of narcoleptics clearly indicates that impaired orexin signaling can cause narcolepsy in humans as well as in animal models.

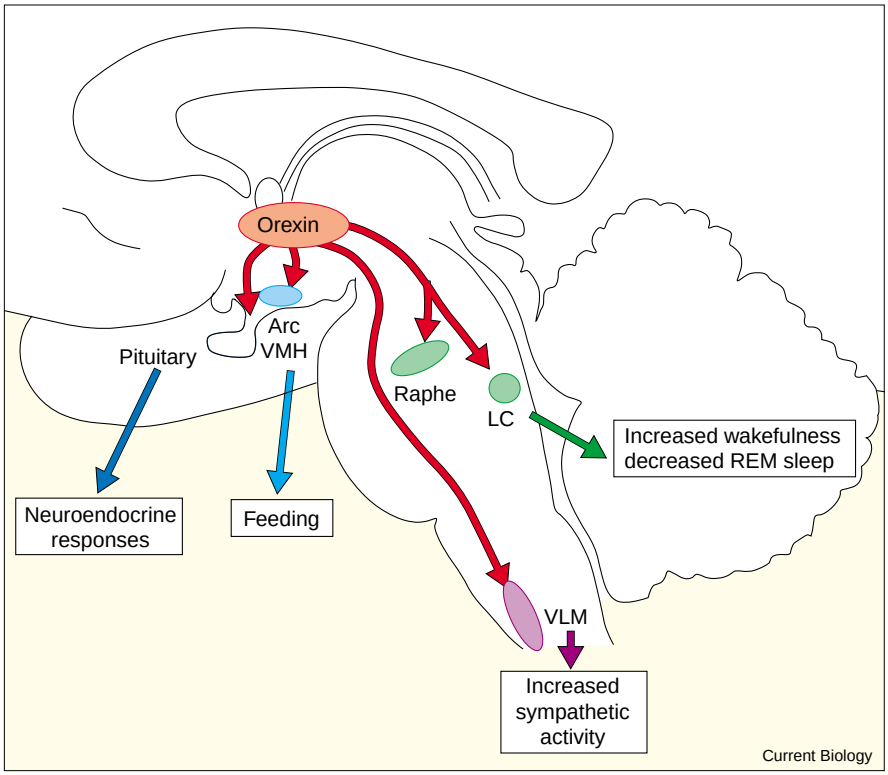
While peptide knockout animals such as the orexin knockout mice are helpful in understanding the role of specific peptides, this approach cannot fully define the function of specific neuronal populations. To produce an animal model that closely resembles human narcolepsy, Hara and colleagues [13] recently produced mice with an acquired loss of orexin neurons. These mice contain a transgene in which the human orexin promoter drives the expression of a mutant form of ataxin-3, a protein that induces apoptosis in neurons. These mice lose 90% of their orexin neurons by 8 weeks of age, and at 15 weeks nearly all orexin neurons are gone. This neuronal death is specific to the orexin neurons, with no apparent loss of nearby neurons such as those that produce melanin concentrating hormone or neuropeptide Y. As seen in orexin peptide knockout mice, adult orexin/ataxin-3 mice have a behavioral phenotype that strongly resembles narcolepsy. The organization of sleep/wake behavior is disrupted, with briefer episodes of wakefulness that may be the functional expression of chronic sleepiness. These mice also have little diurnal variation in the amount of REM sleep, abrupt transitions into REM sleep, and sudden behavioral arrests in which they may wobble or collapse, similar to cataplexy in humans.

How might orexin loss disrupt sleep/wake behavior?

Wakefulness is promoted by aminergic brain regions such as the locus coeruleus which diffusely activate the cortex, and by cholinergic regions that produce thalamic activation, allowing flow of information to and from the cortex. Orexin-containing fibers innervate all these wake-promoting regions, and orexin increases the firing rates of aminergic neurons *in vitro* [5]. Impaired orexin signaling may produce insufficient activation of these wake-promoting areas, resulting in poorly sustained wakefulness.

The dysregulation of REM sleep may be caused by a similar mechanism. Mice normally have 2–3 times as much REM sleep during the day than at night, and they only

Figure 1



Pathways through which orexin neurons control many behaviors. Orexin increases the activity of aminergic arousal regions including the locus coeruleus (LC) and raphe nuclei that then increase wakefulness and suppress REM sleep. Neuroendocrine responses such as increases in cortisol are mediated by projections that influence pituitary function. Orexin neurons may promote feeding through projections to the arcuate nucleus (Arc) and ventromedial hypothalamic nucleus (VMH). Orexin neurons may increase the activity of the sympathetic nervous system through projections to the ventrolateral medulla (VLM) and spinal cord.

enter REM sleep after 8–10 minutes of non-REM sleep. However, both orexin knockout and orexin/ataxin-3 mice exhibit less diurnal variation in REM sleep, and they often enter REM sleep rapidly, sometimes even switching abruptly from wakefulness into REM sleep. People with narcolepsy also can quickly enter REM sleep at any time of day. REM-promoting neurons in the pons are normally inhibited by aminergic neurons, and in the absence of orexin, aminergic activity may be lower and REM sleep may occur more readily. A simple disinhibition of REM sleep seems unlikely, however, because both strains of narcoleptic mice have normal amounts of REM sleep each day. REM sleep is strongly influenced by circadian rhythms [14], and the nearly equal distribution of REM sleep across the light and dark periods in orexin knockout mice suggests that circadian factors may influence sleep/wake behavior through the orexin neurons. Thus, a loss of orexin may disrupt the circadian timing of REM sleep, and this impaired timing would allow REM sleep to occur at any time.

Beyond these abnormalities of behavioral state control, the orexin/ataxin-3 mice also convincingly demonstrate that the orexin neurons influence feeding and energy homeostasis. Mice simply lacking the orexin peptides eat roughly normal amounts, but have a tendency to become obese when fed a high fat diet [15]. In contrast, orexin/ataxin-3 mice become obese even on a normal diet [13]. By 20 weeks of age, they weigh about 40% more than wild-type littermates, but remarkably, they eat less, especially during the night when most feeding occurs.

How might loss of orexin neurons produce hypophagia and obesity?

The phenotypic differences between orexin knockout mice and orexin/ataxin-3 mice may reflect the different background strains in these two models, or it may be that an acquired loss of orexin in young adulthood has different effects than seen with constitutive loss. However, the orexin neurons contain other neurotransmitters in addition to orexin. Chou and colleagues [16] have demonstrated that essentially all orexin neurons contain dynorphin, and loss of the orexin neurons results in a complete loss of dynorphin mRNA in the lateral hypothalamus. Because injection of dynorphin into the hypothalamus promotes feeding, loss of this dynorphin may contribute to the decreased food intake of the orexin/ataxin-3 mice.

A decrease in energy expenditure may explain why loss of orexin neurons produces obesity. Decreased muscular activity may provide a partial explanation, because orexin/ataxin-3 mice have less locomotor activity than normal at night [13]. Basal metabolic rate is strongly influenced by sympathetic

activity, and injection of orexin into the lateral ventricles increases blood pressure, heart rate and plasma epinephrine, most likely through activation of autonomic regulatory brain regions and increases in sympathetic activity [8,17,18]. The thermogenic effects of orexin might be due to sympathetically mediated increases in brown fat heat production. Thus, even at rest, orexin/ataxin-3 mice may have low sympathetic tone, burn fewer calories and become obese.

Do people with a loss of orexin neurons have abnormalities in feeding and metabolism? People with narcolepsy may eat less [19] but are often mildly overweight [20]. While narcoleptics have no gross abnormalities in sympathetic activity, these observations suggest that their basal metabolic rate or level of physical activity may be low, and several studies are underway to define more clearly these metabolic abnormalities.

The orexin/ataxin-3 mice provide a new perspective into the multifunctional nature of the orexin neurons. Loss of orexin clearly produces narcolepsy, but loss of the orexin neurons also produces hypophagia and obesity. These neurons are thus critically positioned to coordinate feeding and the control of autonomic activity and metabolic rate with the regulation of sleep/wake behavior. Integration of these behaviors may help ensure that animals are metabolically active and alert while seeking food at the correct time of day.

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